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STUDIES OF THE RESISTANCE OF WHEAT STARCH TO DIASTATIC ACTION

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JOHN GORDON MALLOCH

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STUDIES ON THE RESISTANCE OF WHEAT STARCH TO DIASTATIC ACTION[1]

By J. G. Malloch[2]

Abstract

The diastatic activity of wheat flour has been definitely resolved into two independent variable factors: (1) concentration and activity of the enzyme, (2) resistance of starch to hydrolysis. The method for the measurement of starch resistance involves the inactivation of the natural diastase with sodium tungstate, washing out the excess precipitant, and the addition of a fixed amount of taka-diastase. By the use of this method it has been found that continued grinding or extraction with ether decreases the resistance of the starch. Both diastatic activity and starch resistance are affected by the conditions under which the wheat is grown. The increase in diastatic activity when wheat is sprouted is due mainly to a change in the quantity or nature of the enzyme. Several modifications in Rumsey's method for diastatic activity are recommended.

Introduction

For the past few years extensive experiments on the effects of environment on the quality of wheat have been conducted by the Department of Field Crops of the University of Alberta. In these experiments the baking test has, of course, been the principal measure of quality. This has been supplemented by other determinations with a view to explaining the results of the baking test and for the purpose of studying the individual factors influencing these results. Among other things, determinations of the diastatic activity of the flour have been made.

There is ample evidence (20, 28, 54, 63) that diastatic activity may have a great influence on the quality of the bread obtained from any flour. It is true that the use of malt preparations in baking practice has somewhat lessened the importance of diastatic activity from a commercial point of view. However, a moderately high natural diastatic activity seems desirable and therefore the environmental factors which may affect the diastatic activity should be studied.

Rumsey's method (63) for the determination of diastatic activity was tentatively adopted for these investigations. It was found necessary, however, to make slight technical modifications and these will be dealt with in a later section of this paper. This method makes use of the determination of the sugar produced in a sample of flour by autolysis under controlled conditions. The results are therefore affected both by the character of the substrate and the nature of the enzyme, as well as by external factors such as the pH.

Until recently cereal chemists have been in the habit of looking on the starch as being essentially the same in all samples of flour. This has led rather commonly to the interpretation of the variation in the results of the Rumsey determination as variation in the activity of the enzyme. That this interpretation is entirely in error is revealed by a review of the literature.

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[2]*Research Assistant, University of Alberta.*

The present investigation was undertaken in order to gather information on the variability of the resistance to diastatic action of the starch from different samples of wheat flour. A method was devised for the measurement of starch resistance which makes it possible to resolve diastatic activity, as determined by the modified Rumsey method, into its two main components, relating respectively to the enzyme and the substrate.

Review of Literature

It is only recently that any attempt has been made to study the properties of wheat starches and to find out the effect of variety and conditions of growth on these properties. For the most part we have to be guided by previous studies which were carried out on starches derived from different species of plants. However, these studies should give clues to those properties in which variations might be expected.

Variation in Physical Properties of Starches.

From the standpoint of susceptibility to enzyme attack, the physical properties of the starch grains may be just as important as their chemical nature, either because of a direct physical effect or because physical differences may indicate differences in composition. Reichert's monumental work (62) is undoubtedly the outstanding research along this line. He was able to establish differences in form, appearance, and size, on the physical side, and differences in temperature of gelatinization, polariscopic properties, and staining reactions, which are probably partly chemical and partly physical, and differences in the chemical behaviour towards various reagents.

The swelling of starch grains has received some attention. Alsberg (5) noted that potato starch differed from most starches in that it disintegrated on boiling. Huss (33) used stains in the study of swelling. He showed that starch grains, changed by pressure, heating, or chemical action, will absorb eosin, congo red, phenol red and a number of other dyes which will not penetrate the uninjured grains.

Marked variation in the size of the granules from different sources has been noticed. Alsberg (5) points out that there is little correlation between the original size of the granule and the size after swelling, owing to the many variables affecting both measurements. The multiplicity of factors affecting granule size affords an explanation of the results obtained by Grewe and Bailey (29) who were unable to demonstrate any relation between size of grains and the quantity of maltose produced by diastatic action in flour. Buchanan and Naudain (17, 53), however, have reported that strong flours contain a higher percentage of small granules than weak flours.

The gelatinization temperature has been investigated by several workers and differences have been found not only between species but even within varieties. Fernbach and Wolff (25) found starch from immature peas to be different in this respect from that of a mature sample of the same variety. La Wall and Graves (37) found differences within varieties of peas and beans. Varieties of maize were found by Dox and Roark (22) to vary in this property.

Alsberg and Rask (2), using a viscosity method, found that gelatinization of wheat and corn starch took place over a range of temperature differing for the two starches.

Rask and Alsberg (60) found significant variations in the viscosity of starch obtained from eleven different flours. Samec (64) reports differences in the viscosity of potato, wheat, corn and rice starches.

Differences have also been found in osmotic pressure (64), specific gravity (86) and other physical properties, but the foregoing review is sufficient to show the variability of the physical nature of starch within one variety as well as between species.

Variation in Composition of Starch.

We now know that starch grains are not composed entirely of carbohydrate material, and that even the carbohydrate portion is not pure starch. Phosphorus, probably in organic combination, is a constituent of starch grains and varies in quantity from species to species. Samec (64) reports variation in the phosphorus content. Malfitano (48) was able to find SiO_2 in starch granules. Lately the fat content of starch has been attracting attention. Taylor and Nelson (81) report its occurrence in corn starch. It has also been found in rice (8), and variations in amount in different samples have been noted.

Ling and Nanji (42) report the presence of hemicellulose in some starches but not in others. Schryver and Thomas (67) found a variation of from 0 to 4% in the hemicellulose content of six starches examined. Clayson and Schryver (19) isolated a hemicellulose from wheat flour and found it identical with that from wheat starch.

There is considerable evidence that starch itself is not a homogeneous substance but is composed of at least two fractions. These have been termed (1) starch cellulose, amylopectin or α-amylose and (2) granulose, amylose or β-amylose. The first set of terms refers usually to the outer portion of the granule and the second to the inner. It must be remembered that, in the present state of our knowledge, the substances to which these terms refer are not very accurately defined, and that some workers (9) hold that the amylopectin is scattered throughout the grain. Nearly a hundred years ago Guibort (30) called attention to the difference between the outer coating and the inner soluble material in the granule. Since that time a great deal of work has been done on this phase of starch chemistry. Bourquelot (14) in 1887 concluded that starch granules are composed of a mixture of carbohydrates. Meyer (51) fully described the preparation of α- and β-amylose. Samec and Haerdtl (64) found that the quantities of amylopectin and amylose in a number of starches, including wheat, maize, rice, and potato, varied considerably. To this they attribute the variation which they found in water content, viscosity and resistance to diastatic action, since Samec and other co-workers (66) were able to show considerable differences in the physical and chemical properties of the amylopectins. Chodat (18) holds that the degree of polymerization of the two fractions may vary from sample to sample and that from this there result differences in behaviour towards diastase. Taylor and Iddles (82)

carried out the separation of α - and β -amylose from a number of common starches and found marked differences in the properties, notably that α-amylose is very slowly hydrolyzed by 10%HCl, while β-amylose hydrolyzes readily to a reducing substance. They also found variations in the amounts present in the starches studied. There were differences between samples of a single species, though not so large as between different species. It is still an open question whether the difference between the two fractions is chemical as a difference in degree of polymerization, or physical, as a difference in degree of hydration. However, the fact remains that starches from different sources vary in practically all the properties that have been studied, and that variations are to be found, though usually to a lesser degree, between samples of starch from the same species of plant. It is not surprising, therefore, that many investigators have found similar variations in the reaction to diastase.

Variations in Resistance of Starch to Diastatic Action.

Unfortunately, much of the work on this phase of the subject has been with gelatinized starches, and the results are thus not strictly applicable to the present problem. The weight of evidence, however, seems to be in favour of the existence of a variation between starches from different species of plants. Ford (27), O'Sullivan (59), and Sherman, Walker and Caldwell (72) all found no significant difference between samples from different sources, using boiled starches, but O'Sullivan noted that a much lower percentage of maltose was obtained from potato starch than from the cereal starches. On the other hand, many papers could be cited, where the opposite results were obtained. The investigations of Levberg (38, 39) and of Lintner (43, 44) in the latter part of the last century fall in this group. Nagao (52) found oat and barley starches were digested more rapidly than wheat or rye starches. Lintner and Baur (45) were of the opinion that rye and barley starches varied in their resistance to diastatic action.

In the present paper, however, the chief interest lies in the evidence for variation in the resistance of the starch in different samples of wheat flour. In a microscopic study of germinating wheat, Whymper (87) showed that there was a variation in the ease with which granules of different sizes were attacked. In 1909 Humphries and Simpson (32) brought forward evidence to show that the rate at which the starch granules are attacked, and not the activity of the enzyme, is the prime factor that determines the gas-making capacity. Rumsey (63), working with one flour and two samples of wheat starch, obtained 247.0, 157.8 and 215.2 mg. of maltose, respectively, produced by the action of 1 gm. of malt extract. Collatz (20) added varying amounts of malt flour to seven samples of flour and one sample of wheat starch. He found that not only were there differences in the amount of reducing sugar produced by the same amount of diastase but that the flours did not maintain the same order as the amount of malt flour was increased. He says, "In general but for one exception the weaker flours produce less reducing sugars than do the stronger when digested with the same amount of malt flour.....From the data presented, it would seem that the starch

of the strong flours was generally more easily converted than that of weak flours." In a later paper, with O.C. Racke (21), he was able to demonstrate that there were substantial differences in the amount of reducing sugars produced by malt extracts in doughs from different flours. Mangels (49) recently reported the results of a study of the factors affecting the diastatic activity of flour. He determined diastatic activity by Rumsey's method, and then used commercial starch as the substrate for the action of a cold-water extract of three of his flours and obtained only slight variations in the amount of maltose produced. The differences in the results of the Rumsey determination, therefore, were due principally to variations in the resistance of the starch. He also subjected three wheat starches to the action of a cold-water extract of Marquis flour, and obtained 44.2, 10.1, and 182.0 mg. of maltose, respectively.

Hermano and Rask (31) working with wheat starches, prepared by the method of Rask and Alsberg (60), and malt diastase, were able to show marked differences between samples of starch obtained from different varieties of wheat grown at different places. It will be seen that the evidence points to the existence of demonstrable differences in the resistance of the starch in different samples of flour to the action of diastase.

Variation in the Action of Diastase.

There is abundant evidence that diastatic enzymes from different sources vary enormously. It is only necessary to mention two studies, which are typical of the bulk of this class of work. Wohlgemuth (88) found that the diastase from various animal sources showed a variation in relative diastatic values of from 10 to 468.8 when a 0.1% starch solution was used as the substrate. Oshima and Itaya (57), working with vegetable diastases, obtained values in amylo-saccharifying units varying from 0 for ungerminated oats and barley to 21,000 for ungerminated soy beans. These authors also studied the variation in the amylo-liquefying and amylo-dextrinizing values. They found that not only did the magnitude of each of these vary but that the ratio between them varied. Their results are in agreement with those of Effront (24) who found that he was able to distinguish between diastatic enzymes from different sources on the basis of the relation of liquefying to sugar-producing power. Numerous other investigators, notably Sherman and his co-workers (70,71) have drawn attention to this dual function of diastase.

The apparent existence of two separate functions has led to the theory that there are two components of diastase or else two distinct but associated enzymes. This was suggested fifty years ago by Maercker (47) and the suggestion has been repeated from time to time since then. Bourquelot (12) is sometimes credited with being the first to propose the theory, but Maercker's publication preceded his by nine years. Until recent years, however, attempts to separate the two enzymes have been largely fruitless. Ohlsson (55, 56) in 1922, and in a more comprehensive paper in 1916, described a method for their separation. The "saccharogen-amylase" is prepared by the destruction of the

other component; this is accomplished by allowing the malt solution to stand for 15 minutes at 0° C. and a pH of 3.3. The "dextrinogen-amylase" is prepared by taking advantage of the instability of the saccharogen-amylase at a temperature of 70°C.

The foregoing discussion shows clearly that variations in sugar production from any starch may be caused by qualitative differences in the enzyme present. That variations may occur as a result of quantitative differences in the enzyme content is a commonplace, and the literature which could be cited in support of it is voluminous.

Very little of the foregoing work has been done with wheat diastase however, so that direct evidence on the problem of the variation, whether qualitative or quantitative, in its sugar-producing power is rather limited. It has already been noted that Mangels (49) found slight differences in the activity of the cold-water extracts of three samples of wheat flour. Thatcher and Koch (85) using several procedures for the water extraction, found a variation in the amylolytic power of the diastase extracted from different grades of flour.

Experimental

The Problem

The literature reviewed in the previous section shows clearly that we may expect variations in the physical and chemical properties of the starch in different samples of wheat flour and a consequent variation in the resistance to diastatic attack. We may also expect to find variations in the sugar-producing power of the diastase, owing to either qualitative or quantitative differences in the enzyme content. It is evident, then, that the values for diastatic activity obtained by Rumsey's method are the resultant of two groups of variable factors. It is admitted that from the point of view of the baker the quantity of maltose produced is the important factor, regardless of whether it is influenced most by the activity of the enzyme or the resistance of the starch. In a study of the effect of environment on wheat quality, however, it is highly desirable that the two groups of factors be studied separately, as it is reasonable to assume the possibility of the resistance of the starch and the activity of the enzyme being influenced differently by environmental conditions.

For a preliminary study on a large scale, such as the one at present in progress at the University of Alberta, it is only necessary to measure one of the sets of factors affecting diastatic activity, and the other can be evaluated by comparison of the results so obtained with the results of the Rumsey determination. It is fully realized that each group of factors is quite complex, and studies with a view to estimating the importance of each of the individual factors in both groups will be undertaken as soon as possible.

In the present investigation it was first necessary to decide whether the starch resistance or the activity of the enzyme could be more easily and accurately measured. Rumsey's review of the literature bearing on this

point was sufficiently convincing to warrant the choice of the former as the most satisfactory line of attack. He shows that it is difficult to obtain a satisfactory substrate. It is possible that this might have been overcome by the preparation of a large quantity of wheat starch, but the extreme difficulty of obtaining a quantitative extraction of the diastase is a grave objection which cannot be so easily avoided. The experiments, therefore, were carried out with a view to finding a method which would measure the resistance of the starch and which would be suitable for routine use on a large number of samples.

It appeared that this could be most effectively accomplished by the adoption of a method in which the natural diastase is rendered inactive and the starch is subjected to the action of a constant quantity of added diastase. Both Collatz (20) and Rumsey (63) carried out experiments on the resistance of starch by adding malt diastase to a flour suspension, determining the total sugar produced and correcting for the original diastatic activity of the flour. While no criticism of this procedure as a method for demonstrating the fact of variation in the substrate is intended, it did not appear to the author to be a satisfactory method for quantitative estimation of the resistance of the starch, where relative results are of prime importance. The possibility of qualitative differences in the diastase in different samples and the lack of any knowledge of the combined effect of diastase from different sources, made it seem advisable to get rid of the natural diastase.

Preliminary Experiments

The preliminary experiments were entirely of a qualitative nature. In the first group the effect of certain volatile substances was tried, with the idea that the flour could be treated and the added substance allowed to evaporate, leaving the flour diastase inactive. These experiments were not very extensive, nor was any great hope entertained for their successful outcome, as in the present state of our knowledge the finding of a suitable substance would be largely a matter of chance. Two compounds were tried unsuccessfully—chloroform and carbon disulphide. In each case marked reduction of Fehling's solution was noted after digestion of the flour. In so far as the chloroform is concerned, this result agrees with the findings of Sherman and Wayman (73). In the same paper, these authors report the inhibiting effect of formaldehyde on diastase. In view of this, a sample of flour was doughed with formalin and allowed to stand over night. The author was acquainted with the denaturing effect of formaldehyde on proteins, but even so he was quite unprepared to find the tough rubbery mass which resulted. Apart from this effect on the proteins, it is doubtful if formalin would have been a suitable substance, because of its action on starch, giving a compound which does not show the iodine reaction (65).

The extraction of the starch by the excellent method of Rask and Alsberg (60) was tried but was deemed unsuitable for several reasons. It is too laborious to be suitable for routine determinations. In addition there is a slight amount

of residual diastase found in the prepared starch and the washing with ether in the course of the preparation is a disadvantage, because of the effect of ether extraction on starch resistance.

The next series of experiments was designed to test the efficiency of inhibiting agents which could be added in solution and the excess of which could be washed out. Sodium tungstate was the first reagent tried, using the procedure for inactivation recommended by Rumsey and getting rid of the excess by washing and centrifuging three times with distilled water. After the third washing, the sample was allowed to digest for one hour at room temperature. A qualitative test with Fehling's solution showed no reduction. This procedure appeared so promising that quantitative experiments were started to determine its suitability.

Inactivation of Natural Diastase.

The procedure followed was similar to that in the qualitative experiments. Ten grams of flour was suspended in approximately 75 cc. of water in a centrifuge tube and immediately inactivated. The suspension was made slightly alkaline, 3 cc. of 15% sodium tungstate was run in and sulphuric acid was added drop by drop until flocculation took place. The tube was then filled to capacity with distilled water and centrifuged. The supernatant liquid was decanted and replaced with distilled water and the mass of flour in the bottom of the tube was stirred up. The tube was then shaken to ensure thorough washing and was recentrifuged. This washing procedure was repeated three times. After the last decantation the flour was transferred to a 200-cc. Kohlrausch flask and suspended in 100 cc. of citrate buffer solution (pH. 4.7 approx.)[1]

The suspension was allowed to stand for one hour at 27°C. The sample was again subjected to treatment with sodium tungstate, centrifuged, and an aliquot taken for determination of reducing sugars by the method of Shaffer and Hartman (69), which was used throughout these experiments.

Some difficulty was experienced owing to the formation of a heavy foam in making the original suspension. On centrifuging, this compacted into a solid layer on top of the liquid, and effectively prevented a clean separation by ordinary decantation. This difficulty was obviated by two means. It was found that if three drops of toluene, which has no effect on diastase (73), are added to the water in the tube before shaking, the amount of foam is much reduced. The removal of the supernatant liquid, after centrifuging, is accomplished by aspirating it out through a small glass tube. It was not considered safe to use caprylic alcohol as an agent for cutting the foam because of a possible effect on the diastase.

[1]*The use of buffer solution instead of distilled water is to make the procedure analogous to our modification of Rumsey's method for diastatic activity. A full discussion of the reasons for adopting this procedure will be found in the section dealing with these modifications.*

It was noticed that after the second washing there was a tendency for the supernatant liquid to become cloudy. The addition of about 15 cc. of the citrate buffer solution to the wash water helps to remedy this condition.

TABLE I

ACTIVITY OF NATURAL DIASTASE AFTER INACTIVATION AND WASHING

Sample	Thiosulphate titration in cc.
Shaffer & Hartmann blank	13.80
Flour No. 1	13.68 13.65
Flour No. 2	13.80 13.80
Flour No. 3	13.85 13.82 13.80
Flour No. 4	13.70 13.70

In Table I it may be seen that the results of all determinations agree with the blank on the reagents within the experimental error of the method. This, of course, means that there were no reducing substances present in the aliquot. The reducing sugars originally present in the flour had all been removed by the washing procedure. The natural diastase of the flour had been completely inactivated and had remained so under the conditions under which the digestion with added diastase would be carried out. The entire absence of reducing sugars is very fortunate, because it obviates the necessity of running a control sample with every determination.

The next step was to see whether or not the washing procedure had completely removed the inactivating agent. When taka-diastase was added to a washed sample, digestion proceeded in a perfectly normal manner. Parke-Davis taka-diastase was selected as the enzyme to be used in these experiments as it is readily available in a fair state of purity. The validity of its use in testing the resistance will be dealt with in a later section dealing with the comparison of flour diastase and taka-diastase.

Procedure for Second Inactivation.

Owing to the presence of the buffer solution, difficulty was experienced in following Rumsey's directions for the use of sodium tungstate after digestion. Since the material to be inactivated has a constant acidity it was deemed allowable to use a fixed procedure for carrying out this step in the process. Experiments were, therefore, performed to determine a procedure which would give satisfactory inactivation and clarification.

A series of 10-gm. samples of flour was freed from natural diastase and transferred to Kohlrausch flasks. To each was added 0.025 gm. of taka-diastase and the sample was immediately inactivated, using 3 cc. of 15% sodium tungstate solution as directed by Rumsey, but varying the amount of N/1 NaOH and H_2SO_4 (1:1) used. Table II gives the thiosulphate titration, after one hour digestion at 27°C., and the efficiency of clarification.

TABLE II

EFFECT OF VARYING AMOUNTS OF ALKALI AND ACID ON INACTIVATION AND CLARIFICATION

N/1 NaOH, in cc.	H_2SO_4 in cc.	Thiosulphate titration, in cc.	Clarification
1	0.6	12.50	good
2	0.6	12.01	good
3	0.6	12.37	very good
4	0.6	12.47	very good
1	1.0	12.52	very good
2	1.0	12.61	very good
3	1.0	12.73	excellent
4	1.0	12.36	excellent
1	1.3	12.61	excellent
2	1.3	12.60	excellent
3	1.3	12.70	good
4	1.3	12.64	good
*Blank		12.70	

**For reducing substances in diastase.*

It will be seen that the best inactivation was obtained by the use of 3 cc. of NaOH and either 1.0 or 1.3 cc. of the acid-water mixture. However, the clarification using 1.0 cc. of acid was markedly better than that when 1.3 cc. of acid was used. A further test using 3 cc. of NaOH and 1 cc. of acid was therefore carried out. The procedure was the same as before but only the one amount of acid and alkali was used. The quantity of taka-diastase was 0.03 gm. instead of 0.025 as in the previous experiment. This accounts for the smaller titration for the blank. Four different samples of flour were used. The results are given in Table III.

TABLE III

TEST OF THE EFFICIENCY OF PROCEDURE FOR INACTIVATION AND CLARIFICATION

Sample	Thiosulphate titration in cc.	Clarification
Flour No. 1	12.52	Excellent
Flour No. 2	12.38	Excellent
Flour No. 3	12.50	Very good
Flour No. 4	12.38	Excellent
Blank	12.52	

All these titrations agree with the blank within the limits of experimental error. All subsequent inactivations in samples containing buffer solution were therefore carried out by adding in succession 3 cc. of N/1 NaOH, 3cc. of 15% sodium tungstate solution, and 1 cc. of H_2SO_4 (1:1).

Description of Flours used.

Before a definite procedure could be adopted it was necessary to test the effect of the variation of several factors on the results obtained. A description of the three flours used in these experiments follows:

Flour A—Milled in the laboratory experimental mill from hard Southern-Alberta Marquis.

Flour B—A commercial patent.

Flour C—A composite flour composed of a mixture of residues of samples of Red Bobs 222 and Marquis milled in the laboratory experimental mill.

Effect of Variation in the Amount of Diastase Added on the Quantity of Reducing Sugar Produced.

It was necessary to get an idea of the amount of reducing sugars that might be expected to result from the digestion with varying amounts of diastase and to establish the type of relationship between amount of enzyme and sugar production.

Ten-gram samples of Flour A (dry weight) were treated according to the procedure outlined in the previous section and digested for one hour at 27°C. with varying amounts of taka-diastase. Blanks containing the same quantities of diastase and buffer solution, but no flour, were run concurrently. The amounts of reducing sugars obtained from the flour were corrected for the amounts found in the blanks. The results are given in Table IV and graphically in Fig. 1.

TABLE IV

EFFECT OF AMOUNT OF TAKA-DIASTASE ADDED ON THE AMOUNT OF SUGAR PRODUCED

Weight of taka-diastase in gm.	Reducing sugar as maltose per 10 gm. of dry flour, in mg.
0.01	73.
0.03	111.
0.05	174.
0.075	214.
0.105	239.
0.15	287.
0.25	372.

As was to be expected, the amount of maltose produced increased with increasing quantity of diastase. This is the common behavior which has been noted by practically every worker in the field, one of the earliest being Schwarzer (68), in 1870.

In 1876 Kjeldahl (35) propounded his "law of proportionality", which holds that the amount of maltose formed is directly proportional to the amount of amylase present, as long as digestion is not more than 40% complete. This

law has received considerable support, including evidence put forward by Ford (26). Its general validity has been questioned, however, by several workers. Ling (40) found that it does not hold for air-dry malt, and Macquaire (46), working with pancreatic diastase, found that there is a relative decrease in the amount of sugar formed per unit of diastase when the amount of diastase is increased.

The amounts of sugar formed in the present series of experiments fall well within the limit of application of Kjeldahl's law. However, it will be noticed that the same increases in the amount of diastase do not give equal

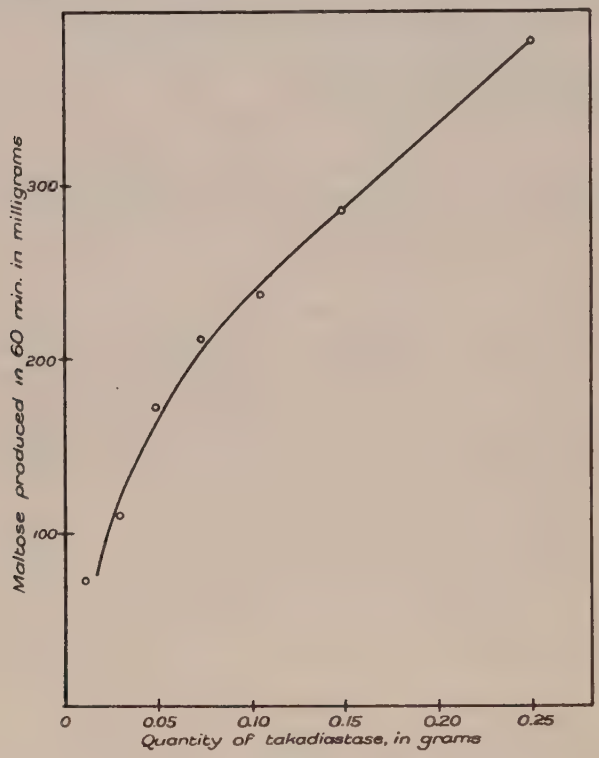

FIG. 1. *Effect of Quantity of Diastase on Amount of Maltose Produced from Ten Grams of Flour (Dry Weight).*

increases in the reducing sugar produced, in all parts of the curve (Fig. 1). Collatz (20) obtained similar curves by the addition of varying quantities of malt flour.

The significance of the curve for the present purpose is that it gives information on which the choice of a quantity of diastase can be based. It is desirable to choose a quantity of diastase from a linear portion of the curve, that is, either less than 0.05 gm. or more than 0.1 gm. of taka-diastase. It was decided to use the lower range, since the quantities of sugar are more easily handled by the Shaffer and Hartman method. The objection that, in this part of the

curve, small variations in the amount of diastase added will give relatively large differences in the amount of reducing sugar, is overcome by the adoption of an accurate technique for measuring out the diastase. A suspension is prepared and aliquots of this used for digestion.

Effect of Time of Digestion on the Amount of Reducing Sugar Produced.

Osterhout in 1918 (59) pointed out the fallacy of measuring the relative rates of biological processes by taking the amount of substance produced in a given time as a measure of that rate. He quite correctly argues that the only true measure of rate where the reaction follows the law for a reaction of the first order is the length of time necessary to produce a given amount. However, in many cases this procedure is most inconvenient, and it is not at all suited to the determination under discussion.

It should be noted, moreover, that Osterhout's calculations are based on the law for a first order reaction. Recently Eadie (23) has found that this formula cannot be applied to diastatic action and that the velocity curve approximates more closely to the formula:

$$v = a + b \log c$$

in which v is velocity, c is concentration of substrate, and a and b are constants.

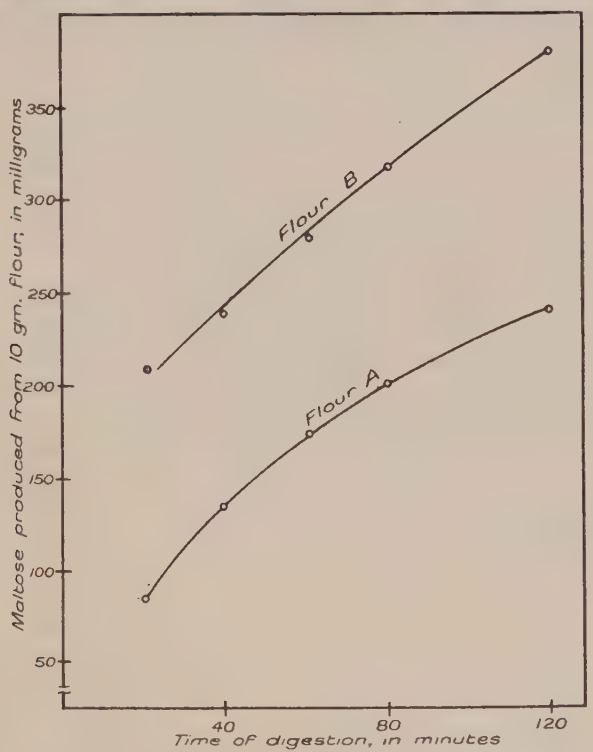

FIG. 2. *Effect of Time of Digestion on the Amount of Maltose Produced by 0.05 gm. Taka-diastase from 10 gm. Flour.*

Brown and Glendinning (16), using a 3% starch solution, concluded that the amount of hydrolysis up to 36% is very nearly a linear function of time, and beyond that point it is approximately logarithmic.

A study of the curves put forward by Osterhout in favour of his contention shows that in the earlier stages of any reaction, while the quantity curves are still rising sharply, the difference in the result obtained by the two methods is not great. It was decided, therefore, to determine the effect of time of digestion on the amount of sugar produced in order to find a procedure which would not be open to criticism on the basis of Osterhout's findings.

Ten-gram samples (dry weight) of flour were used in all these experiments. The flour was inactivated as previously outlined and digested with taka-diastase for varying lengths of time and the results corrected for the taka-diastase blank. The results of the determination of the blank are rather interesting in themselves and a discussion of these will be found at the end of this section. In the first two series 0.05 gm. of taka-diastase was used. The results of these are given in Table V and Fig. 2.

TABLE V

EFFECT OF TIME ON THE AMOUNT OF REDUCING SUGAR PRODUCED BY 0.05 GM. TAKA-DIASTASE ACTING ON 10 GM. SAMPLES OF FLOUR

Time of digestion in min.	Reducing sugar as maltose in mg.		Ratio B/A
	Flour A	Flour B	
20	87	210	2.42
40	134	237	1.78
60	174	280	1.61
80	203	318	1.57
120	240	385	1.61

These data are very similar to those obtained by Rumsey (63) and Collatz (20). An examination of the curves (Fig. 2) shows that both of them, and particularly that for flour A, are from that portion of the whole time curve where the direction is changing. In order to bring the values into the approximately linear portion of the curve it would be necessary to digest for only 20 minutes if 0.05 gm. of diastase is to be used. It is not practical for routine purposes to use a digestion time of much less than 60 minutes. With short digestions, slight, unavoidable variations in the time may affect the result to an appreciable degree. On the other hand, if the time is extended, the number of samples which can be handled in one day may be seriously reduced.

The ratios between the two sets of values given in Table V are rather interesting. For the reason stated above, too much reliance cannot be placed on the results of the 20-minute digestion. The other ratios, and particularly the last three, are suprisingly constant. They constitute an indication that Osterhout's criticism of the method of measuring relative rates by taking the amount produced in a given time may not apply in this case.

Because of the fact that most of the values did not fall on the linear portion of the curve, it appeared that further information was required before a definite procedure could be adopted. Consequently, a similar series of experiments was carried out using 0.03 gm. taka-diastase instead of 0.05 gm. The results of these experiments are given in Table VI and Fig. 3.

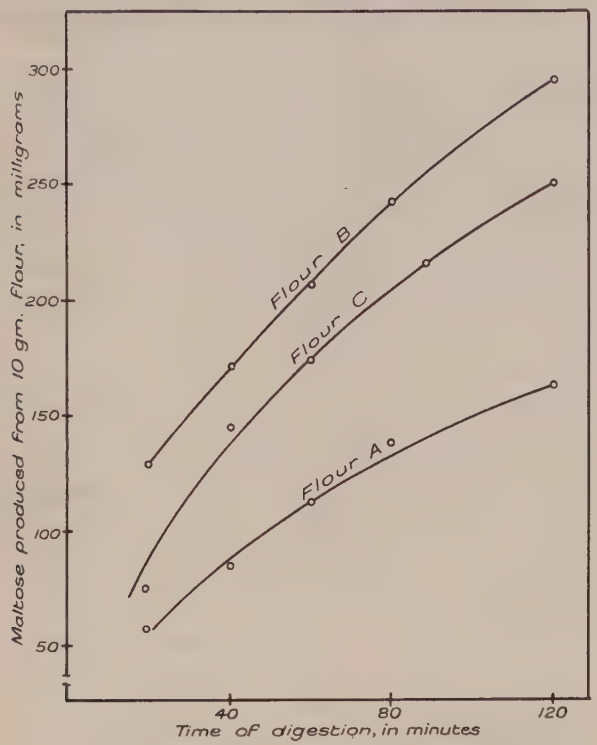

FIG. 3. *Effect of Time of Digestion on the Amount of Maltose Produced by 0.03 gm. Flour from 10 gm. Flour.*

TABLE VI

EFFECT OF TIME ON THE AMOUNT OF REDUCING SUGAR PRODUCED BY 0.03 GM. TAKA-DIASTASE ACTING ON 10 GM. SAMPLES OF FLOUR

Time of digestion in min.	Reducing sugars as maltose in mg.			Ratios	
	Flour A	Flour B	Flour C	B/A	C/A
20	56	129	75	2.03	1.34
40	83	171	145	2.05	1.74
60	111	206	174	1.86	1.56
80	137	243	206	1.81	1.50
120	160	295	250	1.85	1.56

The curves obtained from this series are very similar to those from the previous experiment. The ratios as before show remarkable constancy for digestion times of 60 minutes or more. These results show that 60 minutes

digestion is just as satisfactory as a longer period. In order to decide whether the procedure of measuring the amount of maltose produced in a given time is reliable, it is necessary to compare the data in Table VI with those given in Table V. This can be most easily done by examining the ratios obtained when the amount of sugar produced by 0.05 gm. diastase is divided by the amount produced by 0.03 gm. diastase. These ratios are given in Table VII.

TABLE VII

EFFECT OF TIME OF DIGESTION ON THE RATIO OF SUGAR PRODUCED BY 0.05 GM. TAKA-DIASTASE TO THE AMOUNT PRODUCED BY 0.03 GM. TAKA-DIASTASE

Time of digestion in min.	Ratio of sugar produced by 0.05 gm. and 0.03 gm. taka-diastase	
	Flour A.	Flour B
20	1.55	1.62
40	1.61	1.39
60	1.56	1.35
80	1.48	1.32
120	1.49	1.31

These ratios are also quite constant, and allowing for the difficulty of exact determination for a 20-minute period we can say that the ratio is constant throughout the range studied. Not only is this true but the ratios have values which might be expected from the amounts of taka-diastase used. If the relation between quantity of diastase and amount of sugar produced were a linear function this ratio would be $5/3 = 1.67$. However, we have seen that the relationship is approximately logarithmic in type, consequently the value of the ratio will be slightly lower. This would bring it into agreement with the ratios obtained from the series with Flour A. The fact that the ratios obtained by the use of Flour B are lower can probably be attributed to the fact that the starch of Flour B is more easily converted. While experiments on the effect of quantity of diastase were not carried out with Flour B, we would expect that the curve would show pronounced curvature at lower values than those in the curve for Flour A. The more pronounced the curvature, the lower will be the ratio between the sugars produced by any two quantities of diastase.

The relative rate of hydrolysis with the two quantities of diastase using Osterhout's method (the time necessary to produce a given amount of sugar) was determined. Table VIII shows the ratios which were calculated from the values taken from the common portions of the curves.

TABLE VIII

RATIOS BETWEEN TIMES NECESSARY TO PRODUCE A GIVEN AMOUNT OF SUGAR, USING 0.03 AND 0.05 GM. DIASTASE

Amount of sugar, in mg.	Ratios	
	Flour A	Flour B
100	2.04	—
150	2.10	—
250	—	1.93
290	—	1.85

It will be seen that these ratios are similar to those obtained by the other method, in that they are constant for each flour and that those for Flour B are lower than those for Flour A. However, the values of the ratios are peculiar. It was pointed out above that the value of any such ratio should be 1.67 or less if it is to be a reliable index of the relative activity of the two amounts of added diastase. It is inconceivable that any ratio above that value can give a correct picture of the situation. The statement that the measurement of relative rate in this instance can be more accurately carried out by determining the amount of sugar produced in a given time than by determining the time necessary to produce a given amount of sugar appears to be amply justified.

After consideration of the results reported in this section, it was tentatively decided to use 10-gram samples and to digest for 60 minutes with 0.03 gm. taka-diastase. The final fixing of the procedure would depend on the effect of the size of the sample on the amount of sugar produced.

The Taka-diastase Blank.

In connection with the experiments just reported the amount of sugar produced by the auto-digestion of taka-diastase for varying lengths of time was determined. The diastase was suspended in 100 cc. of the buffer solution and allowed to digest at 27°C. for the lengths of time specified. The enzyme was then inactivated by the sodium tungstate procedure and the reducing sugars determined on an aliquot. The results of two such series are reported in Table IX.

TABLE IX

VARIATION IN THE TAKA-DIASTASE BLANK WITH TIME OF DIGESTION

Time of digestion, in min.	Reducing sugar as maltose in mg.	
	0.05 gm. taka-diastase	0.10 gm. taka-diastase
0	37	80
20	34	79
40	35	79
60	38	80
80	35	78

It can be readily seen that the sugar is present in the taka-diastase before digestion, also that taka-diastase is composed of practically 80% of reducing substance calculated as maltose. It is hardly probable that the reducing substances are all in that form but they must make up a considerable proportion of the weight of the prepared enzyme.[1]

Effect of Concentration of Substrate on Amount of Reducing Sugar Produced.

In this series of experiments the samples were inactivated as usual and digested for one hour with 0.03 gm. taka-diastase. The results of the experiment together with the ratios of the values for the different flours are given in Table X, and the graphs drawn from the data are to be found in Fig. 4.

TABLE X

EFFECT OF CONCENTRATION OF SUBSTRATE ON THE AMOUNT OF REDUCING SUGAR PRODUCED BY 0.03 GM. TAKA-DIASTASE

Dry weight of sample, in gm.	Reducing sugar as maltose in mg.			Ratios	
	Flour A	Flour B	Flour C	B/A	C/A
8	95	174	151	1.83	1.59
10	111	206	174	1.85	1.56
12	130	253	199	1.94	1.53
13	139	265	213	1.90	1.52

The results of these experiments are rather surprising, as it was fully expected that, in view of the excess of substrate, the amount of sugar produced would not vary more than a few milligrams.

The variation is rather difficult to explain without further research. One possible explanation is that the real substrate for the saccharogenic enzyme is liquefied starch and that the production of this is conditioned by the amount of raw starch. It was thought at first that the variation might be due to a dilution effect since the same amount of buffer solution (100 cc.) was used for digestion in each case. However, the results obtained by Swanson and Calvin (79) make this explanation improbable. Fortunately, the ratios of the amounts of sugar produced by different flours at each concentration of substrate show that it makes little difference which of these concentrations are selected, as long as the same amount is used in every determination. It should be remembered, however, that equal amounts of flour do not necessarily mean equal concentrations of starch. The two chief causes of variation in the percentage of starch in flour are variation in the moisture content and variation in the percentage of protein. We have already eliminated the first of these by adopting the dry basis for the weight of all samples. There are two methods of compensating for variation in the amount of protein. We can

[1]*It has since been ascertained that the Parke Davis Co. use maltose to dilute their diastatic preparations.*

weigh out the sample on a constant protein basis or we can correct the results of the determinations to bring them to a constant protein basis. This latter procedure is allowable since the curves obtained from the data in Table X are approximately straight lines. It was decided to adopt 13.5% as the standard protein content, principally because the tables prepared by Shollenberger and Coleman (75) for the conversion of crude protein content to a uniform moisture basis could be used equally well for converting the amount of sugar

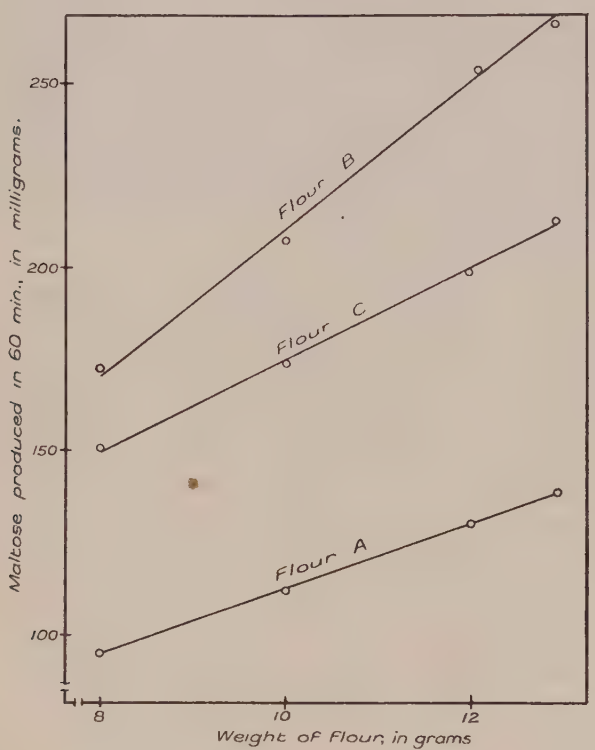

FIG. 4. *Effect of Concentration of Substrate on Amount of Maltose Produced by 0.03 gm. Taka-diastase.*

produced to a uniform protein content basis. It will depend, of course, on the particular experiment whether it will be desirable or necessary to make this correction.

In view of the unexpected result of this experiment, it was considered desirable to determine the effect of variation in substrate concentration, using 0.05 gm. taka-diastase, in order to ascertain whether a similar curve would be obtained. The determinations were carried out in the same manner as in the previous experiment with the exception that a different quantity of diastase was used. The results are given in Table XI.

TABLE XI

EFFECT OF CONCENTRATION OF SUBSTRATE ON THE AMOUNT OF REDUCING SUGAR PRODUCED BY 0.05 GM. TAKA-DIASTASE (FLOUR A)

Dry weight of samples in gm.	Reducing sugar as maltose in mg.	Ratio between sugar produced by 0.05 gm. and 0.03 gm. taka-diastase
8	140	1.48
10	174	1.54
12	201	1.56
13	207	1.48

These results gave the same type of curve as those drawn from the results in Table X. The ratios shown in the table are practically constant and of the same value as those obtained in the experiment on effect of time (Table VII). This was taken as further proof of the statement previously made that the size of sample used is immaterial, as long as the same amount is used in every determination.

METHOD ADOPTED

Ten grams of flour (dry basis) is weighed into a 100-cc. centrifuge tube and a little distilled water poured in on top of it. Three drops of toluene are added and the tube filled about three-quarters full with distilled water. The flour is brought into suspension by shaking. The suspension is then made neutral or slightly alkaline with N/1 sodium hydroxide and the tube shaken to ensure thorough mixing. Three cubic centimetres of 15% sodium tungstate is added, the tube shaken again, and a sufficient quantity of sulphuric acid (1:1) to bring about flocculation is added drop by drop. The tube is shaken again and filled up with water. The suspension is then centrifuged for 4 min. at stop No. 6 (size 2, International centrifuge). The supernatant liquid is removed by aspiration through a small glass tube. About 15 cc. of the buffer solution (pH. 4.7 approx.) is poured in and the mass of flour is loosened from the bottom of the tube with a stirring rod. Distilled water is poured in until the tube is about three-quarters full when the tube is shaken to bring the flour into suspension. The tube is then filled with water and centrifuged as before. This washing procedure is carried out three times. After the last washing, the flour is transferred to a 200-cc. Kohlrausch or other wide-necked volumetric flask, using 95 cc. of buffer solution, at such a temperature that the temperature of the suspension in the flask will be approximately 27°C. The flask is then put in a constant-temperature water-bath set at exactly 27°C. and allowed to remain for about 15 min. to equalize the temperature.

The buffer used is one of Sörensen's citrate-HCl buffers (90). The citrate solution is made up of 21.008 gm. crystalline citric acid plus 200 cc. of N/1 NaOH per litre. The particular buffer used contains 4 parts of citrate solution to 1 part of N/10 HCl, and it is used in a concentration of 1 part of buffer solution to 3 parts of distilled water.

While the suspensions are being brought to temperature a suspension of taka-diastase in buffer solution is made up so that 5 cc. of suspension contain 0.03 gm. taka-diastase. The 5 cc. of this suspension is pipetted immediately into each flask and the time is noted. The digestion is allowed to proceed for exactly 60 min. and the flasks are shaken at intervals during this time. As soon as digestion is completed the suspension is diluted to about 175 cc. with distilled water and inactivated by the addition of 3 cc. of N/1 NaOH, 3 cc. of 15% sodium tungstate and 1 cc. of sulphuric acid (1:1) in succession. The suspension is then made up to volume (200 cc.) and 7 cc. more water added to compensate for the volume of the flour. The flask is shaken till the contents are thoroughly mixed. The sample is then transferred to a centrifuge bottle and centrifuged for 4 min. at stop No. 10 (size 2, International centrifuge). An aliquot (usually 50 cc.) is taken for the determination of reducing sugars by the Shaffer and Hartmann method. Where results reported in the following pages are tabulated as "starch resistance" the numerical values for maltose are converted to their reciprocals, multiplied by 10,000 to avoid decimals. This makes them vary in the same sense as the actual resistance of the starch.

Comparison of Taka-diastase and Extracted Flour Diastase.

It was thought to be desirable to compare the results of the method adopted with those obtained by a similar method, using extracted flour diastase. Accordingly the starch resistance of a series of seven flours (the three experimental flours and four others) was determined by the method adopted. The sugar produced by extracted flour diastase was determined on the same flours. As it was possible to run these determinations on only three flours at one time, the flours were grouped in three series, Flour A being included in each one.

The diastase was extracted from 300 gm. of flour by 1500 cc. of buffer solution for 1.5 hours at room temperature and the extract was centrifuged. The inactivated flour to be digested with flour diastase was suspended in 50 cc. of buffer solution, and 50 cc. of the flour extract—representing the equivalent of 10 gm. of flour—was added to it. The rest of the procedure was the same as in the regular method. The amount of reducing substances in the extract was determined, using 50 cc. of flour extract and 50 cc. of buffer solution. This blank in all cases was quite high owing to the amylolytic activity during the period of extraction.

The reducing sugar produced from Flour A in the three series varied from 99 mg. to 130 mg. While the relation between the activities of the diastase extractions over this range is probably not strictly linear, it was deemed allowable to treat it as such in order to facilitate comparisons. The results of the other two series were reduced to the basis of the activity of the diastase in the first sample. The results as calculated are given in Table XII, and the values for 0.03 gm. of taka-diastase and flour diastase from 10 gm. of flour are plotted against each other in Fig. 5.

TABLE XII

REDUCING SUGAR PRODUCED BY TAKA-DIASTASE AND FLOUR DIASTASE

Sample	Reducing sugar as maltose in mg.	
	0.03 gm. taka-diastase	Flour extract from 10 gm. Flour
A	111	99
B	206	311
C	174	222
D	145	116
E	134	127
F	124	106
G	169	179

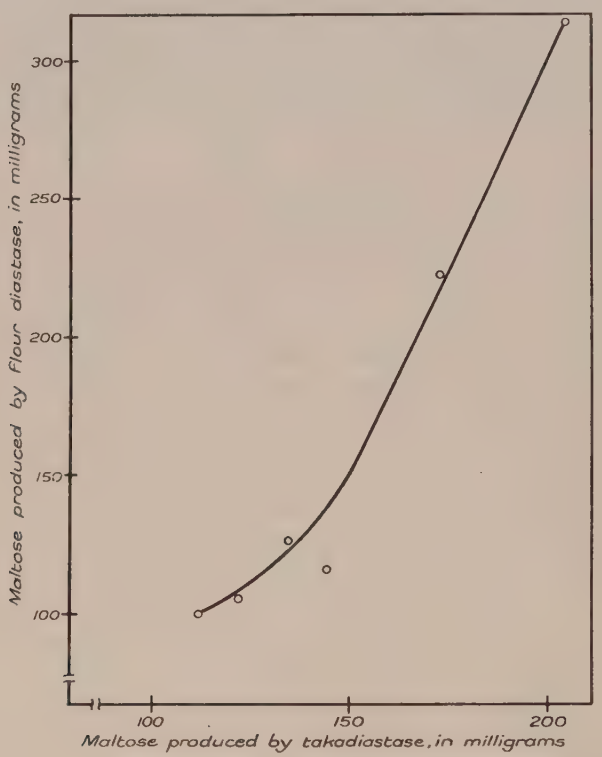

FIG. 5. *Comparison of the Amylolytic Powers of Taka-diastase and Extracted Flour Diastase.*

It will be seen that with the exception of Flours D and E the samples fall in the same order of activity in both series, though the range is greater with the flour extract. The difference between these samples D and E is small, and considering the large possibility of error in the determination with diastase extracted from flour this irregularity cannot be regarded seriously. It seems probable that the value for Flour D with diastase extracted from flour is too low. With the exception of the point for this flour, the values plotted against

each other in Fig. 5 fall very close to a smooth curve. This is a good indication that some definite relation exists between the two sets of values and consequently that the use of taka-diastase in the determination is justified. It may be possible at some later date to determine this relation exactly by running a large number of paired samples, using taka-diastase and flour diastase extracted by a more refined method.

Some Factors Affecting Diastatic Activity and the Resistance of Starch

Alsberg (6, 7) has summarized the factors affecting the diastatic activity of a flour as follows "(1) the numbers of mechanically injured starch grains it contains, (2) the ease with which its uninjured starch grains are attacked, (3) its diastatic content, and (4) the localization and character of its diastase." For the purpose of the investigations now in progress at the University of Alberta, it is more convenient to divide the factors included or implied in the above summary into two groups: (1) properties influenced by the conditions under which the wheat is grown, (2) variation in these properties owing to the milling process. Alsberg's factors 2, 3 and 4, can be included in group 1. In this group also we might include the possibility of other constituents of the flour affecting the resistance of the starch and the activity of the enzyme. Fat is one constituent that might be particularly referred to in this regard. In considering the variation in this group of factors, the possibility of fruitful research on the effect of a number of fundamental influences suggests itself. A few of these are seasonal temperature and precipitation, variety of wheat, stage of maturity at cutting, and damage, such as sprouting, rust, or frost.

In the second group Alsberg makes mention in his summary of only one factor, namely, the proportion of mechanically injured grains. In his discussion, however, he brings out the fact that differences in the fineness of grinding of the flour may have a pronounced effect.

A preliminary study of a few of the points just mentioned was undertaken. As the determination of diastatic activity occupied a prominent place in these studies, it seems advisable before going on to discuss the results of the experiments, to discuss the modifications of Rumsey's method used in this laboratory.

Modification of the Method for Determining Diastatic Activity.

Rumsey found that diastatic activity varied greatly with temperature, and accordingly specified in his method that the temperature of digestion should be exactly 27°C. He also found that diastatic activity was profoundly influenced by the acidity of the flour suspension, the maximum being reached about pH 4.7, but he did not recommend the control of this variable. Sörenson (77) points out the impossibility of obtaining comparative results at varying hydrogen-ion concentration. He suggests that the ideal procedure is to make

determinations at a number of different acidities. However, it appears to the author that where this procedure is not practical, on account of the labor involved, it is desirable to make all determinations in the region of the optimum pH. The easiest method of controlling the acidity is to use a buffer solution. It was necessary, therefore, to find a buffer solution which would give the requisite pH and which was made up of substances which would neither stimulate nor depress the activity of the diastase, apart from their effect on acidity. One of Sörenson's citrate-HCl buffers (90) (citrate solution, 8 parts; HCl 2 parts) was finally chosen. In view of the fact that flour is itself a buffer, it was necessary to determine by experiment whether the desired pH was being obtained. Flour suspensions were made up with 10 gm. of flour in 100 cc. of water or of buffer solution (1 part buffer to 3 parts water) and allowed to extract for 20 min. The liquid was then decanted off and the acidity determined. The results are given in Table XIII.

TABLE XIII

pH OF FLOUR SUSPENSIONS

Sample	Unbuffered	Buffered
Flour No. 1	6.00	4.63
Flour No. 2	5.83	4.65
Flour No. 3	5.93	4.63

These acidities are sufficiently close to pH 4.7 to bring the diastatic activity into the flat portion of the pH activity curve lying on either side of the maximum.

It remained to be determined whether or not sodium citrate had any effect on the diastase independent of the acidity of the solution.

This was done in two ways: by comparison of the activity of a buffered sample with that of one brought to pH 4.7 by the use of lactic acid, and by comparison of samples where the buffer had been used undiluted and where it was used in a 3 to 1 dilution. Any effect of the sodium citrate should be more marked where the buffer is used undiluted. The samples were treated by the usual procedure of digesting for 1 hour, clarifying, making up to 200 cc. and determining the reducing sugars in a 50-cc. aliquot. The results given in Table XIV were corrected for the blank. A sample digested in distilled water is included to show the marked increase in diastatic activity when the acidity is controlled.

TABLE XIV

EFFECT OF SODIUM CITRATE ON DIASTATIC ACTIVITY

Method of controlling acidity	Maltose in 50-cc. aliquot, in mg.
None	31
Lactic acid	54
Citrate buffer (undiluted)	52
Citrate buffer (1 pt. to 3 pts. H_2O)	53

These results show that sodium citrate has no appreciable effect on the diastase. This conclusion is confirmed by results recently obtained by Sherman, Caldwell and Dale (74), working with pancreatic amylase.

In Rumsey's original method, no account is taken of the volume of the flour itself when making the suspension up to volume after digestion. This introduces a slight error. Accordingly the volume occupied by 10 gm. of flour in a number of suspensions was determined. It was found that the average volume was 7 cc. Therefore, the practice has been adopted of allowing for this when diluting the digested suspension. The volume varies slightly from sample to sample so that it is impossible to get an exact volume of liquid but by the addition of an extra 7 cc. the resulting volume is closer to the correct value than it would be if no allowance were made for the volume occupied by the flour.

One of the largest sources of error in the determination of diastatic activity by Rumsey's method has been in the determination of the blank. It was difficult for one analyst or a group of analysts working with different samples of the same flour to check their results. It was, therefore, necessary to find an improved method of determining the blank which would give more reliable results.

Rumsey's directions for the inactivation of the blank are rather ambiguous. After describing the procedure for inactivating the active sample and the preparation of the blank he says "....immediately inhibit diastatic activity by clarifying with the sodium tungstate in the manner just described. The blank determination is then carried out in the same manner except that the addition of 0.4 cc. of concentrated H_2SO_4 is omitted on dilution to volume." In this laboratory sulphuric acid has been used in the clarification of all samples, whether blank or active, as there was no apparent reason for omitting it in the case of the blank. An experiment performed in the course of the present investigation shows that it is fortunate that this procedure was adopted. A sample of diastase-free flour was prepared by the method adopted and 0.1 gm. of taka-diastase added to it. This was treated immediately by Rumsey's procedure, omitting the acid, and allowed to digest for 1 hour at 27°C. At the end of this time 259 mg. of reducing sugar was found. The reducing substances added in the taka-diastase calculated as maltose amount to only 80 mg. It is evident that when the acid is omitted inactivation is not complete.

Theoretically, to be a true blank, the extraction of the natural sugar of the flour should be allowed to proceed under the same conditions as the digestion. Experiments were undertaken to discover whether this procedure would be sound. The first step was to ascertain whether the quantity of reducing sugar in the aliquot increased with time of extraction. A series of 10-gram samples of flour was suspended and inactivated in the usual way (using acid). They were allowed to extract with occasional shaking for varying lengths of time before being made up to volume, centrifuged, and sampled for sugar determination. The time of extraction was taken as the time from suspension to sampling. The results are given in Table XV.

TABLE XV

EFFECT OF TIME OF EXTRACTION ON VALUE OF BLANK

Time of extraction, in min.	Reducing sugar as maltose, in mg.
10	33
30	39
60	40
90	42

It can be seen that there is an increase in the blank with increase in the time of extraction. This is particularly noticeable in the first half-hour, and suggests that part of the variability of the Rumsey blank (using the acid) is due to differences in the time elapsing between the making of the suspension and the determination of the sugars. The difference of 7 mg. in the blank as determined after 10 min. and after the usual digestion period of an hour is sufficient to invalidate the results completely when the diastatic activity of flour is low. The total activity in Rumsey units may not equal more than 50 mg. of maltose.

The next step was to determine whether there was any increase in the copper reduced owing to the hydrolysis either of the starch or of the soluble material by the acid used in inactivation. A large sample of diastase-free starch was prepared by the method outlined in the first part of this paper. This was carried through the usual inactivation procedure and allowed to stand. Portions were taken out at intervals and centrifuged, and 50 cc. aliquots were taken for the determination of sugars.

TABLE XVI

HYDROLYSIS OF STARCH FOLLOWING CLARIFICATION

Time of standing in hr.	Copper from 50 cc. aliquot, in mg.
1	0.95
2	0.95
3	1.02

The figures given in Table XVI show, as was to be expected, that the amount of copper reduced is negligible, and that none of the increase reported in Table XV can be attributed to hydrolysis of the starch.

To determine the amount of hydrolysis of the soluble carbo-hydrates a large sample of flour was suspended, inactivated, and centrifuged immediately. The supernatant liquid was allowed to stand, aliquots for the determination of sugars being taken at intervals. The amounts of copper reduced are given in Table XVII.

TABLE XVII

HYDROLYSIS OF SUPERNATANT LIQUID FOLLOWING CLARIFICATION

Time of standing in hr.	Copper from 50 cc. aliquot, in mg.
0.	8.74
0.75	8.61
1.00	8.80
2.00	9.70
26.00	17.67

It will be seen that hydrolysis does proceed but that the effect is negligible during the first hour. We must conclude, therefore, that the increase reported in Table XV is due to the increased extraction of the sugar present in the flour.

Procedure for Determining the Blank in Diastatic Activity Determinations.

The procedure adopted for determining the blank in diastatic activity determinations is as follows: The sample is suspended in distilled water, inactivated according to Rumsey's directions for inactivating the active sample (with acid) and extraction is allowed to go on for 60 min. at 27°C. An aliquot is taken and the reducing sugars are determined immediately. A test of this method was made by determining the blank on four 10-gm. samples of one flour. The results of this test are given in Table XVIII and show much better agreement than was possible using the old procedure. The modifications outlined in this section were used in all the diastatic activity determinations reported in this paper.

TABLE XVIII

QUADRUPLICATE DETERMINATIONS OF BLANK BY METHOD ADOPTED

Sample	Reducing sugars as maltose, in mg.
1	27
2	26
3	25
4	27

Effect of Fineness of Grinding on Resistance of Starch to Diastatic Action.

Through the courtesy of Dr. T. A. Pascoe of the University of Minnesota the writer was able to obtain a very fine series of samples for this study. The flours were from three different wheats, one set of samples (E) being milled in the laboratory experimental mill and the other set (P) in the Minnesota State experimental mill. Thus we have three pairs of flours from the three wheats, differing in fineness of grinding. One of the flours (401P) was further ground for 4, 8 and 20 hours in a ball mill, giving three more degrees of fineness. The figures for starch resistance are given in Table XIX.

TABLE XIX

EFFECT OF FINENESS OF GRINDING ON STARCH RESISTANCE

Sample	Starch Resistance
387E	94
387P	40
392E	78
392P	53
401E	72
401P	37
401P (ground 4 hrs. in ball mill)	30
401P (ground 8 hrs. in ball mill)	28
401P (ground 20 hrs. in ball mill)	24

It will be seen that each of the three flours showed increased susceptibility to enzyme attack as the fineness was increased. Alsberg (3, 4) has pointed out that the increase in diastatic activity on grinding may be due to an increase in the number of damaged grains or to the splitting up of the flour particles which allows the enzyme easier access to the substrate. Ziegenspeck (89) also directed attention to the packing of the granules into lumps being a possible factor in starch resistance. In the present experiment, this factor is probably more important than injury to the granules, since even if there were extensive injury any soluble material would be removed by the washing during the determination, so that any increase in the extractable fraction of the starch owing to grinding would have no influence on the result of the determination. Moreover, a microscopic examination of Flour 401P, ground for twenty hours in the ball mill, showed very few starch grains that would stain with congo red, which they should do if there was any injury to the outer layers (33). This observation agrees with the results obtained by Shollenberger and Coleman (76). They found that the percentage of solids soluble in cold water decreased rather than increased in the finer separates of normal flour. There could not, therefore, have been any appreciable proportion of injured grains.

Effect of Extraction with Ether on the Diastatic Activity and Starch Resistance.

The researches of Taylor and his co-workers (81, 83) have shown that there is a certain amount of fat intimately associated with the starch granule. The concentration of this fat was shown to vary by Aoi (8), who also showed that the extraneous fat associated with starch varied in amount. In view of the fact that lipoids have a marked effect on surface tension and that absorption probably plays an important part in enzyme action (1, 10, 11) it is altogether likely that fats in both forms affect the diastatic action. The extraneous fat may have another effect, for, as Ziegenspeck (89) has pointed out, the permeability for diastase of the layers surrounding the starch grain plays an important role in digestibility.

It has been shown by Taylor and Nelson (81) and by Rask and Phelps (61) that ordinary ether extraction will not remove the lipoids which are combined in the granule. It is, therefore, possible to investigate the two classes of lipoids separately. It was decided for the time being to confine the experiments to the extraneous fat.

The first step was to determine the effect of ether on the diastatic activity and starch resistance without extracting any of the lipoids. This was done by moistening the flour with ether and allowing it to evaporate. The experiment on the effect of this treatment on diastatic activity was carried out with Flour H, but unfortunately samples of this flour were not available for the other experiments reported in this section. Flour A was used for studying the effect of treatment with ether on the starch resistance.

TABLE XX

EFFECT OF TREATMENT WITH ETHER ON DIASTATIC ACTIVITY AND STARCH RESISTANCE

Treatment	Diastatic activity	Starch resistance
None	208	90
1 hour in ether	220	—
24 hours in ether	—	74
48 hours in ether	227	—

It will be seen in Table XX that simple contact with ether lowered the resistance of the starch and increased the value of the sample in Rumsey units. While these changes might be caused by a direct effect of the ether on the enzyme, it is more probable that they are due to an altered distribution of the lipoids. As the ether evaporates it brings the fats to the surface of the mass of flour, and even though the samples were shaken up repeatedly during the evaporation, a change in the location of the fat must have taken place.

In the next experiment, reported in Table XXI, samples of Flours A and B were extracted with ether for 24 hours in Soxhlet's apparatus and diastatic activity and starch resistance were determined on both the extracted and unextracted flour. In weighing out the samples, allowance was made for the amount of fat extracted.

TABLE XXI

EFFECT OF ETHER EXTRACTION ON DIASTATIC ACTIVITY AND STARCH RESISTANCE

Sample	Diastatic activity	Blank	Starch resistance
Flour A	143	40	90
Flour A (extracted)	177	49	70
Flour B	242	61	48
Flour B (extracted)	286	61	35

With both flours the resistance of the starch to diastatic action was lowered and the diastatic activity increased. It has been pointed out that lecithin (36, 84) and certain of the oleates (78) may have an inhibiting effect on diastase. However, it is improbable that this phenomenon affects the results to any marked degree. The increase in diastatic activity in the experiment reported in Table XX must have been due to some other factor, as these substances were not removed. Moreover, the increase in diastatic activity in the second experiment (Table XXI) is not greater than we would expect from the results of the starch resistance determination.

Recently Johnston (34) reported an experiment on the effect of ether extraction on diastatic activity. He found that in every case the extracted flours gave higher values. In discussing his results he says, "As the flour particles were much finer after extraction, it is suggested that the starch in the natural flour may exist in relatively large clumps of granules held together by fatty material. When this fatty material is removed the clumps fall apart, thereby forming a more suitable substrate for the diastase". This suggestion is supported by the results of the starch resistance determinations reported in Table XXI.

Johnson's statement in his summary, that the "reducing sugar content was higher in the ether-extracted flours than in the corresponding natural flours", is misleading, and must be interpreted in the light of two sentences in his discussion: "The suggestion that the removal of fatty material from flour allows the clumps of starch to fall apart also explains the higher reducing-sugar content of the extracted flour as compared with the natural flour. The clumps of starch may occlude reducing sugars which are extracted for determination only when the occluding material falls apart."

Johnson does not describe his method of extracting the reducing sugars, but it is assumed from his method of reporting the results that he accepted the results of the Rumsey blank as a measure of this constituent in the flour. The results given in Table XV show that the blank of the diastatic activity determination, whether determined according to Rumsey's directions or by

the modification outlined in this paper, is not a reliable measure of the reducing sugar content of the flour. This is especially true when Rumsey's directions are followed.

The values of the blanks obtained by the modified method are reported in Table XXI. The most interesting feature of these results is that with Flour A extraction increased the blank while in Flour B the blanks were identical. This can probably be explained by the difference in granulation of the natural flour. Flour A, which was milled in the laboratory mill, has a much coarser granulation than Flour B (a commercial patent). After extraction, the granulation appeared to be very similar, in the A and B flours, and not markedly finer than the original commercial patent. Alsberg and Griffing (3) report that the increase by continued grinding, in the percentage of the flour constituents extracted by cold water, is due principally to the increase in reducing substances extracted. We would expect, therefore, that changes in granulation from any cause would lead to an increase in the extractable reducing sugars. Since there is a marked change in granulation with Flour A, there is a marked change in the blank. With Flour B, although there is a change in the character of the particles on extraction, the fineness is not greatly affected and consequently there is no change in the blank.

Effect of Environment, Variety, and Stage of Maturity at Cutting on Diastatic Activity and Starch Resistance.

A series of flours from wheat grown in connection with another project was available for this study. After cutting, all these samples were carefully protected from weathering. The results are given in Table XXII. The figures for starch resistance were corrected for the protein content of the flour.

TABLE XXII

EFFECT OF PLACE OF GROWTH, VARIETY AND MATURITY OF WHEAT ON DIASTATIC ACTIVITY AND STARCH RESISTANCE

Lab. No.	Place grown	Variety	Stage of maturity at cutting	Diastatic activity	Starch resistance
ERB1	Edmonton	Red Bobs 222	Slightly green	222	51
ERB8	Edmonton	Red Bobs 222	Mature	193	52
EM1	Edmonton	Marquis	Slightly green	208	56
EM8	Edmonton	Marquis	Mature	181	62
SM1	Saskatoon	Marquis	Slightly green	173	54
SM8	Saskatoon	Marquis	Mature	161	51
SG1	Saskatoon	Garnet	Slightly green	189	53
SG8	Saskatoon	Garnet	Mature	219	45

Effect of Environment. This is shown by comparison of EM1 with SM1 and EM8 with SM8. In both cases it will be seen that while the diastatic activity is lower in the Saskatoon samples the starch is more easily attacked. This indicates that the activity of the enzyme is greater in the Edmonton-grown samples.

Effect of Variety. Study of the corresponding 1 and 8 samples of two varieties at each place gives us this comparison. The diastatic activity and starch resistance vary inversely with each other. Thus it appears that the difference in diastatic activity between the two varieties is due principally to differences in the resistance of the starch.

Effect of Stage of Maturity at Time of Cutting. This is shown by a comparison of the "1" samples with the corresponding "8" samples. In the Edmonton series the diastatic activity decreases and the resistance of the starch increases with the delay in cutting. The resistance of the starch is apparently the main factor affecting the result of the Rumsey determination recorded as diastatic activity. With the Saskatoon series the results are quite irregular. In both varieties the starch in the mature sample is more susceptible to enzyme attack. In spite of this, the mature Marquis shows a lower diastatic activity than the corresponding green sample, indicating that there must have been a marked decrease in the activity of the enzyme.

Recently Mangels and Stoa (50) reported an extensive study on the effect of stage of maturity at cutting on the quality of Marquis wheat. They concluded that the diastatic activity showed a tendency to decrease towards maturity. The results from three out of the four pairs of samples studied here are in agreement with this. Bracken and Bailey (15) have studied the effect on wheat of standing in the field after ripening, and report that the diastatic activity shows little change. Tascher and Dungan (80) working with corn have found that the activity of the extracted diastase decreases as the grain approaches maturity.

On account of the small number of samples employed, it is not possible to draw any general conclusion as to the effect of any of the factors on diastatic activity or starch resistance. The data, however, support the findings of Hermano and Rask (31) and Mangels (49) that variations in these properties exist between varieties and between samples of the same variety grown at different places. The results emphasize the necessity of studying the starch resistance as well as the diastatic activity in investigations involving the growing of a number of varieties at different places.

The Effect of Sprouting on Diastatic Activity and Starch Resistance.

A quantity of Red Bobs 222 wheat was divided into four samples, one of which was milled without further treatment and the other three were sprouted for varying lengths of time before being milled. The diastatic activity and starch resistance are given in Table XXIII. The starch resistance figures were corrected for protein content.

TABLE XXIII

EFFECT OF SPROUTING ON DIASTATIC ACTIVITY AND STARCH RESISTANCE

Description of sample	Length of sprouts in inches	Diastatic activity	Starch resistance
Unsprouted	—	146	75
Short sprouts	0–¼	391	63
Medium sprouts	¼–¾	552	69
Long sprouts	¾–1½	834	70

The increase in diastatic activity on sprouting is so well known as to need no comment. The results of the starch resistance determination are peculiar. The starch resistance decreases and then rises again as sprouting progresses. This initial decrease is in agreement with Ling's work (41) with raw and malted barley, but any attempt to explain this behavior would at present be mere speculation. The increase in the values after sprouting has started is somewhat easier to understand. Whymper (87) noticed that during germination some of the grains were readily attacked and almost disappeared before others showed any marked breaking down. It may be that some at least of the more readily hydrolyzable starch is removed during the period of sprouting and that the resistance of the remaining starch is thereby enhanced.

In any event, the differences in the starch resistance are not great, especially when they are compared with the differences in diastatic activity. There is no doubt that the increase in the values obtained by Rumsey's method is due to an increase in the amylolytic power of the enzyme content.

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REFERENCES

1. Alexander, J. Some colloid chemical aspects of digestion, with ultra-microscopic observations. J. Amer. Chem. Soc. 32: 680-687. 1910.
2. Alsberg, C. L. and Rask, O. S. On the gelatinization by heat of wheat and maize starch. Cereal Chem. 1:107-115. 1924.
3. Alsberg, C. L. and Griffing, E. P. Effect of fine grinding upon flour. Cereal Chem. 2:325-344. 1925.
4. Alsberg, C. L. and Perry, E. E. Further studies on the effect of fine grinding on starch grains. Proc. Am. Soc. Biol. Chem.; J. Biol. Chem. 63:lxvi-lxvii. 1925.
5. Alsberg, C. L. Studies upon starch. Ind. Eng. Chem. 18: 190-193. 1926.
6. Alsberg, C. L. Starch in flour. Cereal Chem. 4: 483-492. 1927.
7. Alsberg, C. L. The role of starch in bread making. Paper in "A Comprehensive Survey of Starch Chemistry" compiled and edited by R. P. Walton, Chemical Catalog Co., New York. 1928.
8. Aoi, T. R. On the fat in the starch granules of rice. (Translated Title). J. Chem. Soc. Japan. 44:755-763. 1923.
9. Bakhuyzen, H. L. Van De Sande. Starch grains of wheat considered as partially dehydrated amylose. Proc. Soc. Exptl. Biol. Med. 23: 195-197. 1925.
10. Bayliss, W. H. On some aspects of adsorption phenomena with special reference to the action of electrolytes and to the ash constituents of proteins. Biochem. J. 1:175-229. 1906.
11. Bayliss, W. M. The properties of colloidal systems: II. Adsorption as preliminary to chemical reaction. Proc. Roy. Soc. (London) (B) 84:80-98. 1911-12.
12. Bourquelot, E. Ann. inst. Pasteur 1:337. Cited by Ohlsson. 1887.
13. Bourquelot, E. Sur quelques points relatifs à l'action de la salive sur le grain d'amidon. Compt. rend. 104: 71-74. 1887.
14. Bourquelot, E. Sur la composition du grain d'amidon. Compt. rend. 104:177-180. 1887.
15. Bracken, A. F. and Bailey, C. H. Effect of delayed harvesting on quality of wheat. Cereal Chem. 5:128-146. 1928.
16. Brown, H. T. and Glendinning, T. A. The velocity of hydrolysis of starch by diastase with some remarks on enzyme action. J. Chem. Soc. 81: 388-400. 1902.
17. Buchanan, J. H. and Naudain, G. G. Influence of starch on strength of wheat flour. Ind. Eng. Chem. 15: 1050-1. 1923.
18. Chodat, R., Ross, J. W. and Phila, M. The specificity of starches. Chem. Abstracts 19:2756. 1925.
19. Clayson, D. H. F. and Schryver, S. B. The hemicelluloses: I. The hemicellulose of wheat flour. J. Biochem. 17:493-496. 1923.
20. Collatz, F. A. Flour strength as influenced by the addition of diastatic ferments. Amer. Inst. of Baking Bull. 9. 1922.
21. Collatz, F. A. and Racke, O. C. Effects of diastase and malt extract in doughs. Cereal Chem. 2:213-227. 1925.
22. Dox, A. W. and Roark, G. W. The determination of gelatinization temperatures of starches by means of an electrically heated chamber on the microscope stage. J. Amer. Chem. Soc. 39:742. 1917.
23. Eadie, G. S. The effects of substrate concentration on the hydrolysis of starch by the amylose of germinated barley. Biochem. J. 20:1016-1023. 1926.
24. Effront, J. Sur les propriétés distinctives des amylases de différentes provénances. Compt. rend. 174: 18-21. 1922.

25. Fernbach, A. and Wolff, J. Analogie entre l'amidon coagule par l'amylocoagulase et l'amidon de pois. Compt. rend. 140:1547. 1905.

26. Ford, J. S. Note on the hydrolysis of starch by diastase. J. Chem. Soc. 85:980-983. 1904.

27. Ford, J. S. and Guthrie, J. M. On the identity of the hydrolytic products of starches of various origins. J. Soc. Chem. Ind. 24: 605-606. 1905.

28. Ford, J. S. and Guthrie, J. M. The amylolytic and proteolytic ferments of wheaten flours and their relation to "baking value". J. Soc. Chem. Ind. 27:389-393. 1908.

29. Grewe, E. and Bailey, C. H. The concentration of glutenin and other proteins in various types of wheat flour. Cereal Chem. 4:230-247. 1927.

30. Guibort. Extrait d'un mémoire sur l'amidon. Ann. chim. phys. 40:183-193. 1829.

31. Hermano, A. J. and Rask, O. W. A consideration of certain reactions of starches with special reference to enzyme hydrolysis. Cereal Chem. 3:361-393. 1926.

32. Humphries, A. E. and Simpson, A. G. Gas-making capacity as a factor in the estimation of strength of wheaten flours. 7th Int. Cong. of App. Chem. Trans. Sect. 6a, p. 27-38. J. Soc. Chem. Ind., 28:738. 1909.

33. Huss, H. Untersuchungen über die Quellung der Stärke. Arkiv. für Botanik 18:1-23. Botan. Abstracts 13:428-429. 1922.

34. Johnson, A. H. Studies of the effect on their breadmaking properties of extracting flours with ether. Cereal Chem. 5:169-180. 1928.

35. Kjeldahl, J. Undersgelser over sukkerannende Fermenter. Meddelelser fra Carslberg Lab. Bind 1:107-184. 1876-82.

36. Lapidus, H. Diastase und Handelslecithin. Biochem. Z. 30:39-55. 1911.

37. La Wall, C. H. and Graves, S. S. Studies in carbohydrates. The composition and digestibility of wheat bread and allied foods. Gelatinization of starches. Trans. Wagner Free Institute of Sciences. Philadelphia. 7:41. 1910.

38. Levberg and Georgiyesky. Sur l'action produite par la saline sur différentes espèces d'amidon, et sur l'influence des acides dilués sur la propriété saccharifère de la saline. Bull. soc. chim. nouv. série 25:393-394. 1876.

39. Levberg and Georgiyesky. Différences observées entre les amidons de diverses origines soumis à la réaction diastatique. Bull soc. chim. nouv. série 26:452. 1876.

40. Ling, A. R. Malt analysis. Federated Institutes of Brewing Journal. 8:441-456. Also Z. ges. Brauw. 25:473-476. 1902.

41. Ling, A. R. Action of diastase on the starch granules of raw and malted barley. Brit. Assoc. Advancement Sci. Rep. 603-4. 1903.

42. Ling, A. R. and Nanji, D. R. Studies on starch: Pt. 1. The nature of polymerized amylose and amylopectin. J. Chem. Soc. 123:2667-2688. 1923.

43. Lintner, C. J. Zur Einwirkung von Diastase auf unverkleisterte Stärke. Chem. Zentr. Bd. 1:500. 1890.

44. Lintner, C. J. Zur Einwirkung von Diastase auf unverkleisterte Stärke. Woch. schr. Brau. 7:310. 1890.

45. Lintner, C. J. and Baur, A. Ueber ein zu 40% ausgemahlenes Gerstenmehl. Z. angew Chem. 36: 469-471. 1923.

46. Macquaire, P. Essais de la diastase amylolytique du pancréas. Compt. rend. 158: 1289-1291. 1914.

47. Maercker, M. Standpunkt unserer Kenntniss über die diastatischen Vorgänge. Chem. Zentr. 559. 1878.

48. Malfitano, G. and Catoire, M. L'amylocellulose considérée comme composé d'acide silicique et d'amylose. Compt. rend. 174:1128-1130. 1922.

49. MANGELS, C. E. Factors affecting the diastatic activity of wheat flour. Cereal Chem. 3:316-323. 1926.

50. MANGELS, C. E. and STOA, T. E. Effect of stage of maturity on composition and quality of Marquis wheat. Paper read at Convention of Assoc. of Cer. Chemists, 1928, and reported in N. W. Miller 154(9):1059. Also Cereal Chem. 5:385-94. 1928.

51. MEYER, A. Untersuchungen über die Stärkekörner. Wesen und Sebens-geschichte der höheren Pflanzen. Jena. Verlag von Gustav Fischer. 1895.

52. NAGAO, Y. Vergleichende Studien über die Einwirkung von Pankreasdiastase auf Hafer-und Weizenstärke. Z. exptl. Path. Therap. 9:227-237. 1911.

53. NAUDAIN, G. G. Study of the properties of wheat starch and the baking qualities of flour. Am. Food J. 20:250-257. Chem. Abstracts, 19:2245. 1925.

54. NEUMANN, M. P. and SALECKER. Ueber den Einfluss diastasereicher Malzpräparate auf die Backresultate. Landw. Jahr. 37:857-872. 1908.

55. OHLSSON, ERIK. Sur l'existence de deux ferments amylolytiques dans la diastase du malt. Compt. rend. soc. biol. 87:1183-1184. 1922.

56. OHLSSON, ERIK. The two components of malt diastase. Compt. rend. trav. lab. Carlsberg. 16:1-68. 1926.

57. OSHIMA, K. and ITAYA, S. Studies on the amylases of different origin for alcoho brewing. J. Agr. Chem. Soc. Japan 1:455-476. 1925.

58. OSTERHOUT, W. J. V. Note on measuring the relative rates of life processes. Science 48:172. 1918.

59. O'SULLIVAN, J. A comparison of the products of hydrolysis of potato starch with those obtained from cereal starches. J. Chem. Soc. 85:616-623. 1904.

60. RASK, O. S. and ALSBERG, C. L. A viscometric study of wheat starches. Cereal Chem. 1:7-26. 1924.

61. RASK, O. S. and PHELPS, I. K. Extraction and estimation of lipoids in cereal products. Ind. Eng. Chem. 17:187-189. 1925.

62. REICHERT, E. T. The differentiation and specificity of starches in relation to genera, species, etc. Stereochemistry applied to protoplasmic processes and products and as a strictly scientific basis for the classification of plants and animals. Carnegie Inst. Wash. Pub. 173. 1913.

63. RUMSEY, L. A. The diastatic enzymes of wheat flour and their relation to flour strength. Am. Inst. Baking Bull. 8. 1922.

64. SAMEC, M. and HAERDTL, H. Studien über Pflanzenkolloide. 9. Zur Kenntnis verschiedener Stärkearten. Kolloidchem. Beihefte. 12:281-300. 1920.

65. SAMEC, M. and MAYER, A. Studien über Pflanzenkolloide. 10. Über die Einwirkung von Formaldehyde auf Stärke. Kolloidchem. Beihefte. 13:165-192. 1921.

66. SAMEC, M., MINAEFF, M. and RONZIN, N. Studien über Pflanzenkolloide. 15. Ueber die aus verschiedenen Stärkearten dargestellte Amylopektine. Kolloidchem. Beihefte. 19:203-212. 1924.

67. SCHRYVER, S. B. and THOMAS, E. M. The hemicelluloses:2. The hemicellulose content of starch. Biochem. J. 17:497-500. 1923.

68. SCHWARZER, A. Ueber die Umwandlang der Stärke durch Malzdiastase. J. prakt. Chem. Bd. 109:212-230. 1870.

69. SHAFFER, F. A. and HARTMANN, A. F. The iodometric determination of copper and its use in sugar analysis. J. Biol. Chem. 45:349-390. 1921.

70. SHERMAN, H. C. and SCHLESINGER, M. D. A comparison of amyloclastic and saccharogenic powers. J. Am. Chem. Soc. 35:1784. 1913.

71. SHERMAN, H. C. and THOMAS, A. W. The influence of certain acids and salts upon the activity of malt amylase. J. Am. Chem. Soc. 37:623. 1915.

72. Sherman, H. C., Walker, F. and Caldwell, M. L. Action of enzymes upon starches of different origin. J. Am. Chem. Soc. 41:1123-1129. 1919.

73. Sherman, H. C. and Wayman, M. Effect of certain antiseptics upon the activity of amylases. J. Am. Chem. Soc. 43:2454-2461. 1921.

74. Sherman, S. C., Caldwell, M. L. and Dale, J. E. A quantitative study of the influence of sodium acetate, sodium borate, sodium citrate and sodium phosphate upon the activity of pancreatic amylase. J. Am. Chem. Soc. 49:2596-2598. 1927.

75. Shollenberger, J. H. and Coleman, D. A. Tables for converting crude protein and ash content to a uniform moisture base. U.S. Dep. Agr. Misc. Circ. 28:1-30. 1924.

76. Shollenberger, J. H. and Coleman, D. A. Influence of granulation on the chemical position and baking quality of flour. U.S. Dep. Agr. Bull. 1463:1-35. 1926.

77. Sörenson, S. P. L. Hydrogen-ion concentration in bread making. Am. Food. J. 19: 556-560. 1924.

78. Stocks, P. Experiments on the action of unsaturated fatty acids and lipoids on amylolytic and hemolytic phenomena. J. Path. & Bact. 23:1-14. 1919-20.

79. Swanson, C. O. and Calvin, J. W. A preliminary study on the conditions which affect the activity of the amylolytic enzymes of wheat flour. J. Am. Chem. Soc. 35:1635-1643. 1913.

80. Tascher, W. R. and Dungan, G. H. Seedling vigor and diastatic activity of dent corn as related to composition of endosperm and stage of maturity. J. Am. Agron. Soc. 20:133-142. 1928.

81. Taylor, T. C. and Nelson, J. M. Fat associated with starch. J. Am. Chem. Soc. 42:1726. 1920.

82. Taylor, T. C. and Iddles, H. A. Separation of the amyloses in some common starches. Ind. Eng. Chem. 18:713-717. 1926.

83. Taylor, T. C. and Lehrman, L. The unsaturated fatty acids associated with corn starch. J. Am. Chem. Soc. 48: 1739-1743. 1926.

84. Terroine, E. F. Lecithin und diastatische Wirkungen. Biochem. Z. 35:506. 1911.

85. Thatcher, R. W. and Koch, G. P. The quantitative extraction of diastases from plant tissues. J. Am. Chem. Soc. 36:759-770. 1914.

86. Thoerner, W. Die Werwendung der Centrifuge bei analytischen und mikroskopischen Arbeiten. Untersuchung und Unterscheidung verschiedener Mehlsorten und Stärkearten mit Hilfe der Centrifuge. Chem. Ztg. 15:1201-1203. 1891.

87. Whymper, R. Microscopical study of changes occurring in starch granules during the germination of wheat. Proc. 7th Int. Cong. App. Chem (Starch Section). London. Also J. Soc. Chem. Ind. 28: 806. 1909.

88. Wohlgemuth, J. Grundriss der Fermentmethoden. Berlin. J. Springer. 1913.

89. Ziegenspeck, H. Starch. Botan. Arch. 7:251-273. Chem. Abstracts, 20:1647. Botan. Abstracts 14:893. 1924.

90. International Critical Tables, Vol. 1, McGraw-Hill Book Co., New York.

VITA

Author—John Gordon Malloch.

Born—Edinburgh, Scotland, September 14, 1900.

Academic History—Graduated from Victoria High School, Edmonton, Alberta, Canada, in 1917. B.Sc. 1924, University of Alberta. Entered the Graduate School, University of Minnesota, June 1925; received the Ph.D. degree in September 1928, major Agricultural Biochemistry, minor Organic Chemistry.

Present Address—National Research Council of Canada, University of Alberta, Edmonton, Alberta, Canada.